

## INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

REC'D 13 OCT 2004



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Applicant's or agent's file reference Case 21415	<b>FOR FURTHER ACTION</b> See Notification of Transmittal of International Preliminary Examination Report (Form PCT/PEA/416)	
International application No. PCT/EP 03/10574	International filing date (day/month/year) 23.09.2003	Priority date (day/month/year) 27.09.2002
International Patent Classification (IPC) or both national classification and IPC C12P23/00		
Applicant DSM IP ASSETS B.V. et al.		

- This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.
- This REPORT consists of a total of 6 sheets, including this cover sheet.  
  
☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).  
  
 These annexes consist of a total of sheets.

- This report contains indications relating to the following items:
  - ☒ Basis of the opinion
  - ☐ Priority
  - ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
  - ☐ Lack of unity of invention
  - ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
  - ☐ Certain documents cited
  - ☐ Certain defects in the international application
  - ☐ Certain observations on the international application

Date of submission of the demand  11.03.2004	Date of completion of this report  14.10.2004
Name and mailing address of the international preliminary examining authority:   European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. +31 70 340 - 2040 Tx: 31 651 epo nl Fax: +31 70 340 - 3016	Authorized Officer  Gruber, A  Telephone No. +31 70 340-8997  

**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT**

International application No. **PCT/EP 03/10574**

**I. Basis of the report**

1. With regard to the **elements** of the international application (*Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17)*):

**Description, Pages**

1-12 as originally filed

**Claims, Numbers**

1-8 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).  
☐ the language of publication of the international application (under Rule 48.3(b)).  
☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.  
☐ filed together with the international application in computer readable form.  
☒ furnished subsequently to this Authority in written form.  
☒ furnished subsequently to this Authority in computer readable form.  
☒ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.  
☒ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:  
☐ the claims, Nos.:  
☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

*(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)*

6. Additional observations, if necessary:

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**V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability;  
citations and explanations supporting such statement**

**1. Statement**

Novelty (N)	Yes: Claims	1-8
	No: Claims	
Inventive step (IS)	Yes: Claims	
	No: Claims	1-8
Industrial applicability (IA)	Yes: Claims	1-8
	No: Claims	

**2. Citations and explanations**

**see separate sheet**

**Re Item V**

**Reasoned statement with regard to novelty, inventive step or industrial applicability;  
citations and explanations supporting such statement**

1 The following documents (D) are referred to in this communication:

D1: WO 94 06918 A (GIST BROCADES NV) 31 March 1994

D2: US-A-5 744 341 (CUNNINGHAM JR FRANCIS X ET AL) 28 April 1998

2 The present application describes a process for producing zeaxanthin and  $\beta$ -cryptoxanthin which comprises cultivating a recombinant microorganism which is expressing a  $\beta$ -carotene hydroxylase gene (*crtZ*) and belonging to the genus *Xanthophyllomyces* (*Phaffia*) and isolating the resulting carotenoids from the cells of said recombinant microorganism or from the cultured broth.

3 The subject-matter of claims 1 - 8 is new in the sense of Article 33(2) PCT.

4 However, the present application does not meet the criteria of Article 33(1) PCT, because the subject-matter of claims 1 - 8 does not involve an inventive step in the sense of Article 33(3) PCT.

4.1 Document D1, which is considered to be the closest prior art, discloses (the references in parentheses applying to this document) the industrial application of *Phaffia* for carotenoid production (page 1, line 22 - page 2, line 29), and suggests that transformation of *Phaffia* with the *crtZ* gene can be used for the increased production of carotenoids, e.g. zeaxanthin (page 9, line 36 to page 10, line 6). In addition, the conditions for cultivation of the latter microorganism are disclosed (page 10, lines 30 - 36; page 11, lines 1 - 2; pages 12 - 17; page 21, lines 7 - 8).

Therefore, the disclosure of document D1 can be considered as a suggestion that the person skilled in the art would take as an incentive to transform indeed *Phaffia* with the *crtZ* gene. By doing so, the person skilled in the art would arrive at a result falling within the scope of claim 1 with positive attitude and with a reasonable expectation of success, requiring nothing extraordinary, all being a matter of technical convenience.

In addition, the process defined in claim 1 differs from that disclosed in document D1 in that it is used not only for the production of zeaxanthin but also  $\beta$ -cryptoxanthin.

The objective technical problem to be solved by the present invention may therefore be regarded as to provide a microbiological process to produce zeaxanthin and  $\beta$ -cryptoxanthin.

The solution to this problem proposed by claim 1 consists of a process employing a microorganism of the genus *Xanthophyllomyces* (*Phaffia*) expressing  $\beta$ -carotene hydroxylase.

Document D2 describes the production of zeaxanthin and  $\beta$ -cryptoxanthin by a microorganism that produces carotenoids and that was transformed with the  $\beta$ -carotene hydroxylase gene from *A. thaliana* (col. 5, lines 35 - 39; col. 6, lines 37 - 45). Document D1 discloses that *Phaffia*, a microorganism that produces carotenoids, can be transformed with the *crtZ* gene in order to increase the production of carotenoids, e.g. zeaxanthin (page 9, line 36 to page 10, line 6).

From the teaching of document D2 it would be obvious to the person skilled in the art that a process, as described in document D1, can be used for the process according to claim 1. The subject-matter of claim 1 does therefore not involve an inventive step (Article 33(3) PCT).

- 5 Dependent claims 2 - 8 do not appear to contain any additional features which, in combination with the features of any claim to which they refer, meet the requirements of the PCT with respect to inventive step.

Neither the use of a certain *Xanthophyllomyces* sp., the source of the *crtZ* gene, the use of "control sequences" nor specific process parameters, as defined in claims 2 - 8, make the subject-matter of claims 2 - 8 inventive because these features are known and well established in the technical field.

- 6 The subject-matter of claims 1 - 8 is susceptible of industrial application (Article 33(4) PCT).

- 7 Aside from the above-mentioned objections, the application does not meet the requirements of Article 6 PCT, because claims 2, 5, and 6 are not clear.

A lack of clarity objection has to be made with respect to the term "mutant" of claim 2. Mutants of microorganisms are obtained on the basis of a random process, therefore, in the absence of any functional limitation, or any further specification of the term, the skilled person is not enabled to identify univocally the subject-matter

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associated with said term.

The term "substantially homologous" in claim 5, prevents the claimed subject-matter from being unambiguously distinguished from the prior art with respect to novelty and inventive step (Guidelines C-III 4.5a).

The term "the control sequence" in claim 6 is unclear and does not allow to define the subject-matter of the claims precisely.